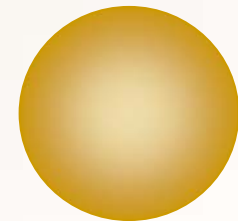


PRE-IMPLANTATION GENETIC DIAGNOSIS AND ASSISTED REPRODUCTIVE TECHNOLOGY IN HAEMOPHILIA

DR PENELOPE FOSTER



MELBOURNE IVF

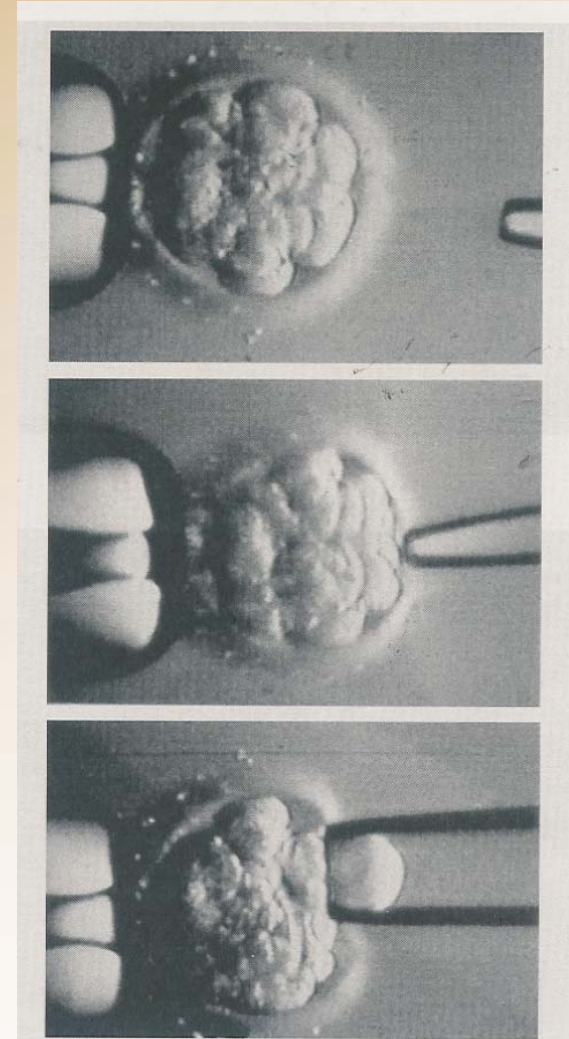
WHAT IS PGD ?

- early embryo diagnosis
- allows selection of unaffected embryos for transfer to patient
- alternative to antenatal testing and termination of affected pregnancy



TECHNIQUE OF PGD

- standard IVF cycle
- biopsy of 1 or 2 cells from day 3 embryo
- diagnostic testing on biopsied cells
- selection of embryos for transfer



PGD IN HAEMOPHILIA

OPTIONS

Sex selection:

- if affected husband, all male offspring unaffected, all females carriers = select male embryos for transfer
- if carrier wife, 1/2 males affected, 1/2 females carrier = select female embryos for transfer



PGD IN HAEMOPHILIA

OPTIONS

Specific gene detection

- avoids discarding unaffected male embryos
- avoids transfer of carrier female embryos



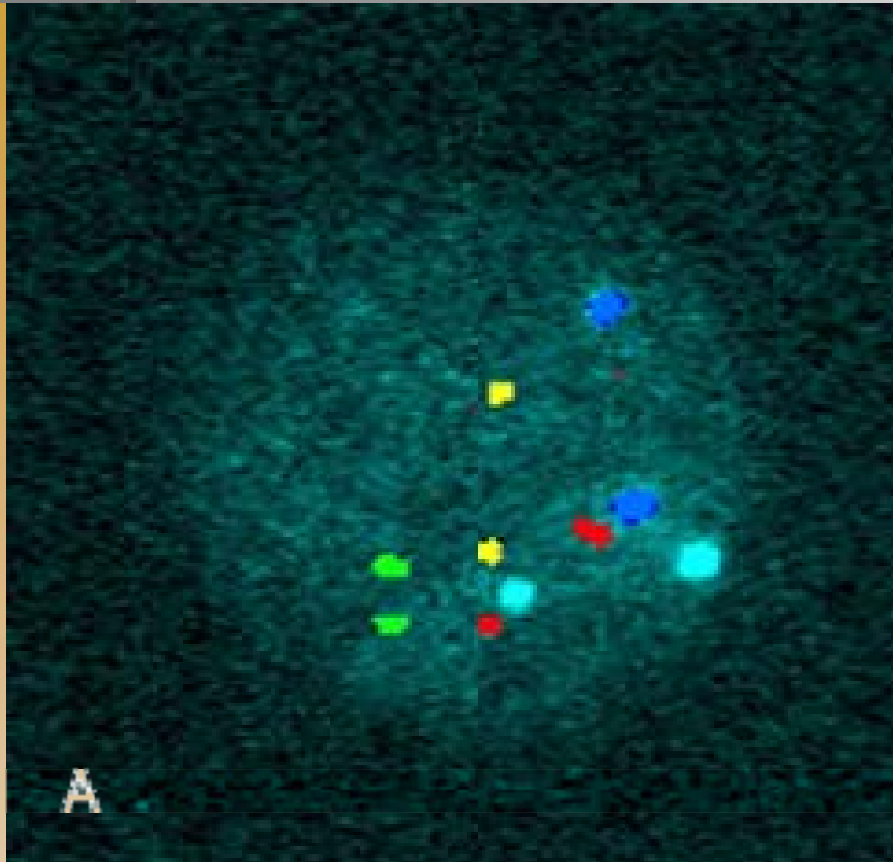
SEX SELECTION - FISH

FLUORESCENT IN-SITU HYBRIDISATION

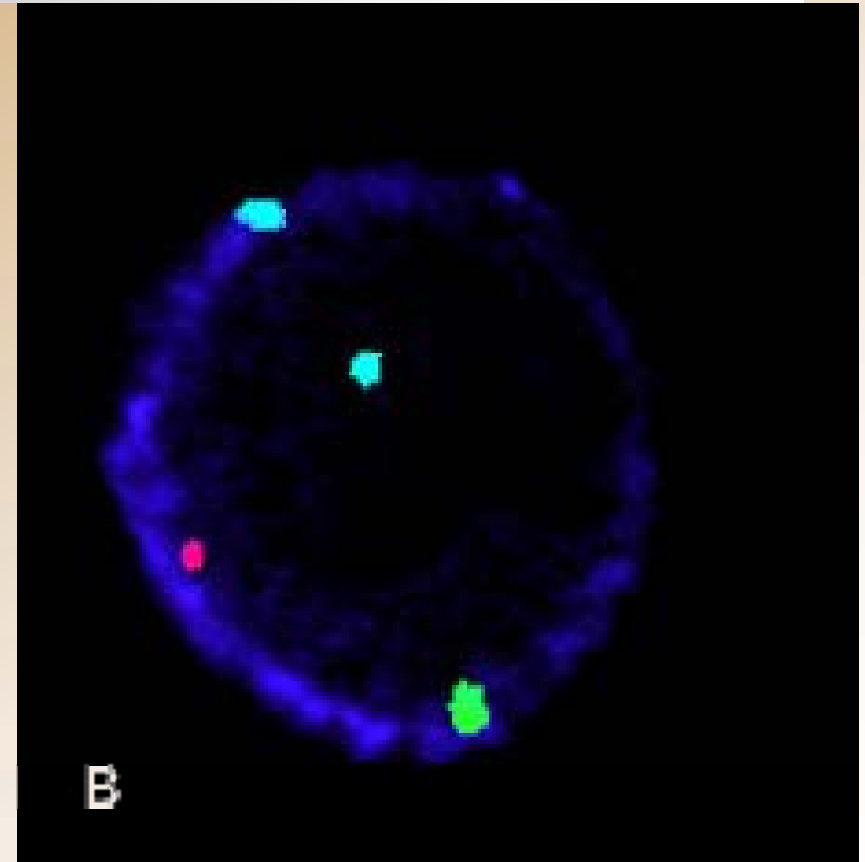
- detects chromosome number
- cell from embryo fixed to slide
- apply FISH probes
- DNA sequences complementary to small segment of particular chromosome
- probes labelled with coloured fluorochromes
- coloured spots indicate presence of sequence
- 8-probe FISH – chromosomes 4,13,16,18,21,22,X,Y
- select euploid XX or XY embryos for transfer



FISH ON BLASTOMERES

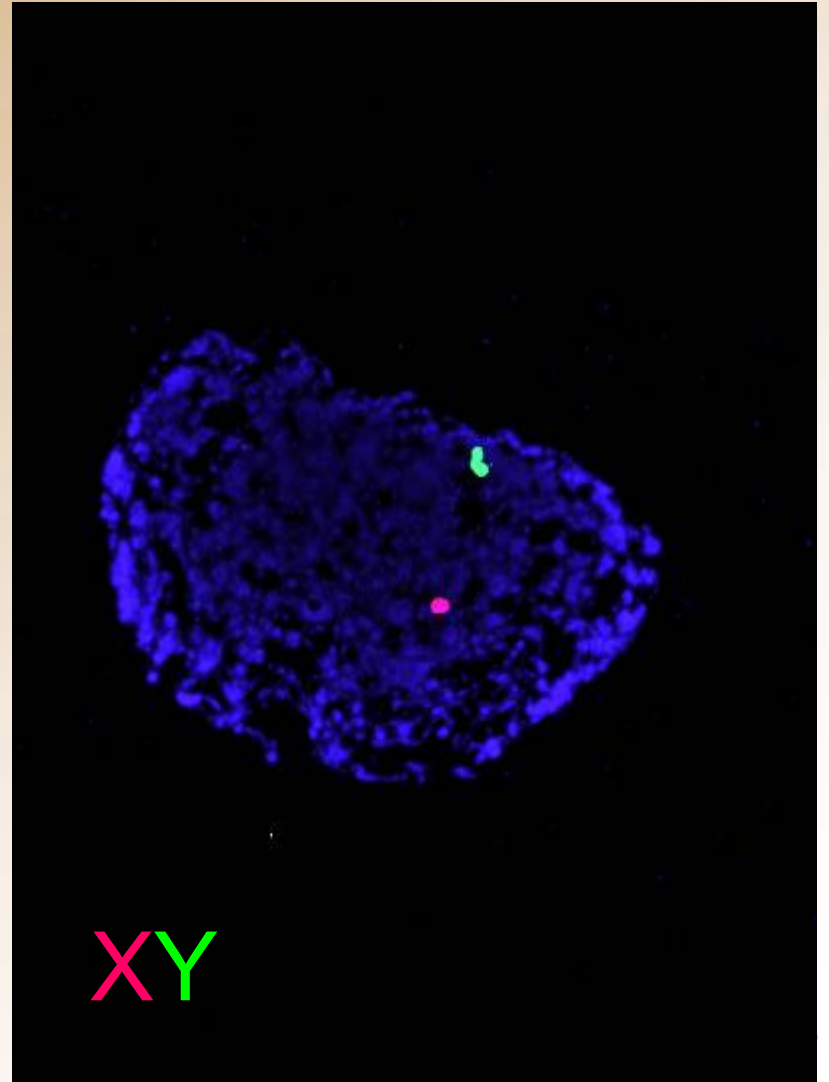
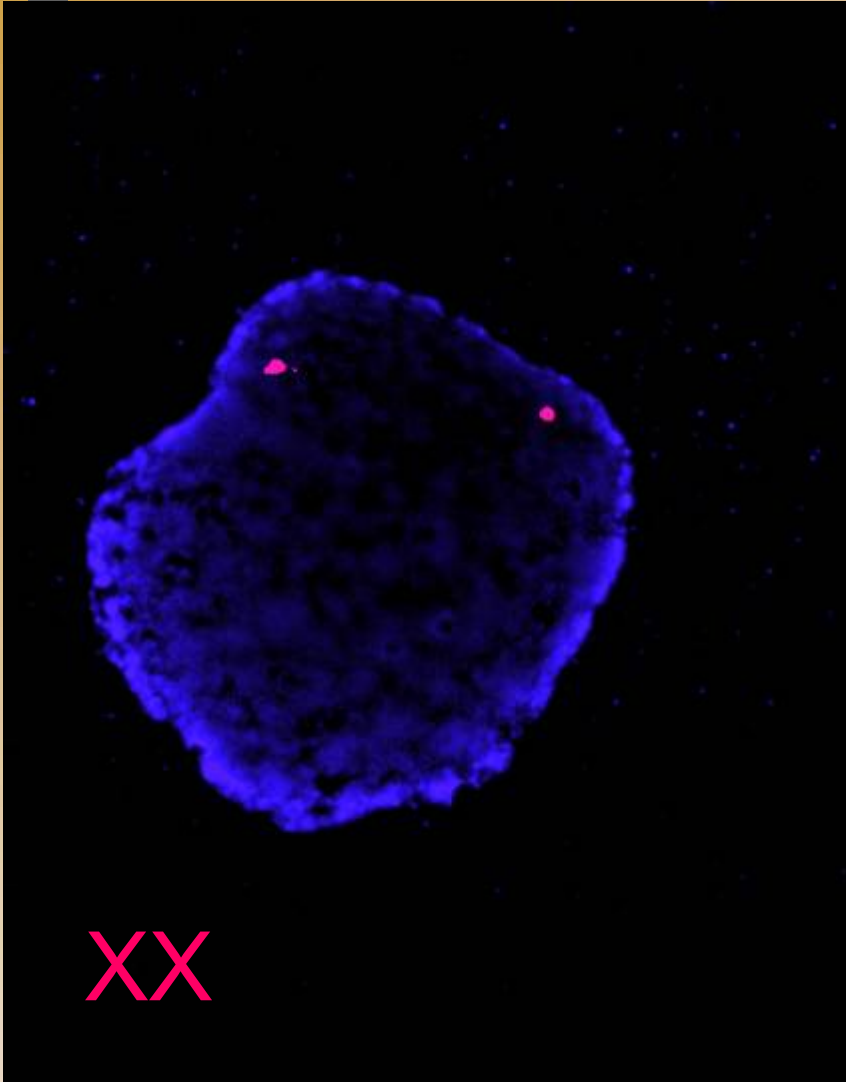


13, 16, 18, 21, 22



X, Y, 4





PGD FOR SPECIFIC GENE DETECTION

- DNA amplification by PCR
- 2 cells from embryo
- fragment analysis on DNA sequencer
- inclusion of informative markers
- individualised tests for each couple
- significant time and effort required for each test



Add title

Husband



Mother



| Markers | Alleles |
|-------------|---------------|
| DXS1073 | 124, 126, 128 |
| DXS8061 | 139, 145, 147 |
| Factor VIII | wt, mut |

AMEL 111 =

X c.some

117 =

Y c.some

Unaffected
Female



128
147
wt



124
145
wt

Unaffected
Male



124
145
wt



Carrier
Female



128
147
wt



126
139
mut

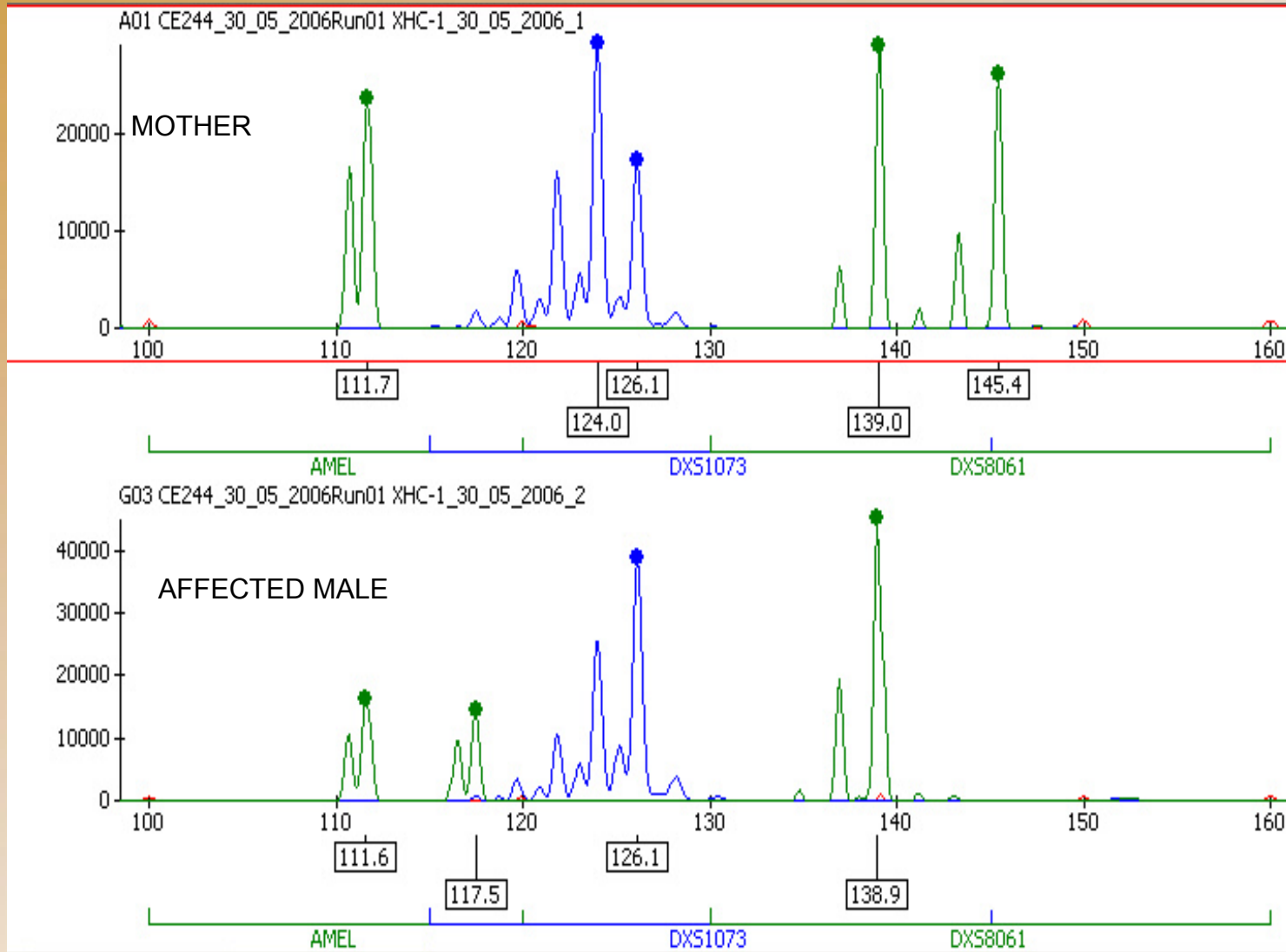
Affected
Male



126
139
mut



RESULTS OF PCR ANALYSIS FOR HAEMOPHILIA A



MONOGENIC PGD AT MELBOURNE IVF

Tests developed to date:

- Cystic fibrosis
- β -thalassaemia
- α -thalassaemia
- Duchennes muscular dystrophy
- α -1-antitrypsin deficiency
- Kennedys disease
- Fragile-X
- Motor neurone disease (exclusion)
- Huntington's disease (direct)
- Huntington's disease (exclusion)
- Neurofibromatosis 1
- Hirschprung's disease
- X-linked hydrocephalus
- Myotonic dystrophy
- Chronic granulomatous disease
- Niemann-Pick type C
- Opitz syndrome
- Rapp-Hodgkin ectodermal dysplasia

Multiple cases for many of these

Tests being developed:

- Congenital adrenal hyperplasia
- Tuberous sclerosis
- Multiple exostosis

Waiting:

- BRCA2
- Waardenburg syndrome
- Neurofibromatosis 2
- AR polycystic kidney disease
- Treacher-Collins syndrome
- Menkes disease
- Familial adenomatous polyposis
- Retinoblastoma
- WHIM syndrome
- Haemophilia A



Conditions that have been diagnosed by PGD – worldwide

- Cystic fibrosis
- Tay Sachs disease
- β -thalassaemia
- Sickle cell anaemia
- Rh blood typing
- Spinal muscular atrophy
- Adrenogenital syndrome
- Congenital adrenal hyperplasia
- Plakophilin-1 (PKP1)
- MCAD
- CDG1C
- Epidermolysis bullosa
- Gaucher's disease
- Hyperinsulinemic hypoglycemia PHH1
- Fanconis anemia
- HLA matching
- Fragile X
- Myotonic dystrophy
- Huntingtons
- Wiscott-Aldrich syndrome
- Incontinentia pigmenti
- Ornithine transcarbamyase def.
- Myotubular myopathy
- Hunter syndrome
- Fabry disease
- Choroideraemia
- Kallman syndrome
- Coffin-Lowy syndrome
- Barth syndrome
- Hypospadias
- Golabi-Rosen syndrome
- Marfans syndrome
- Charcot-Marie-Tooth disease (type 1A)
- Amyloid polyneuropathy
- Crouzons syndrome
- NF2
- Osteogenesis imperfecta I and IV
- Stickler syndrome
- Tuberous sclerosis
- Central core disease
- Familial adenomatous polyposis coli
- Li Fraumeni syndrome
- Lesch Nyhan syndrome
- Duchenne muscular dystrophy
- Becker muscular dystrophy
- Haemophilia A
- Charcot-Marie-Tooth disease
- Retinitis pigmentosa
- Ornithine Transcarbamyase Deficiency
- Agammaglobulinemia
- Alport syndrome
- Hunter's syndrome MPSII
- Oro-facial-digital syndrome type 1
- Adrenoleukodystrophy
- Chronic granulomatous disease
- Menkes disease
- Lowe syndrome
- Ectodermal dysplasia
- Epilepsy
- BRCA1
- Ataxia
- Renal agenesis
- Norrie disease



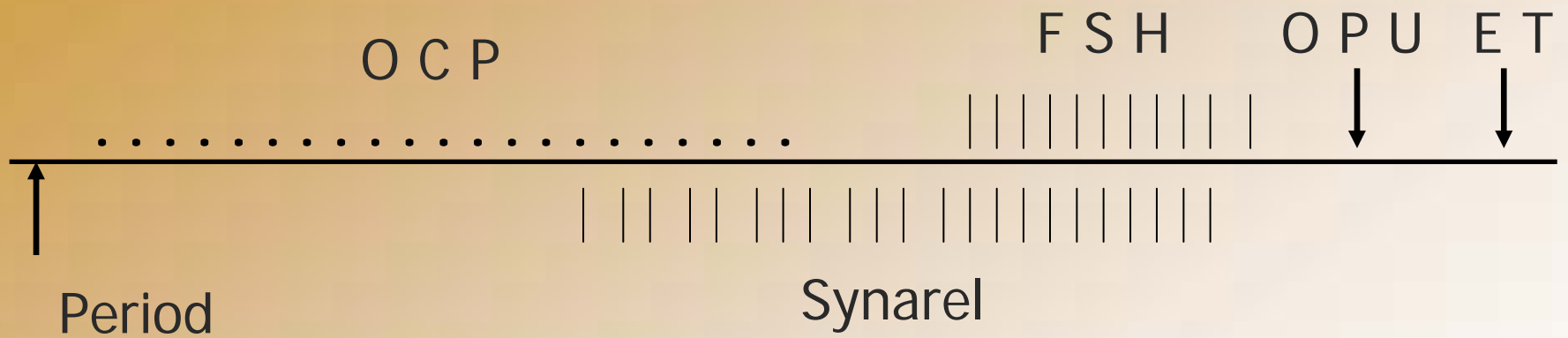
IVF Cycle

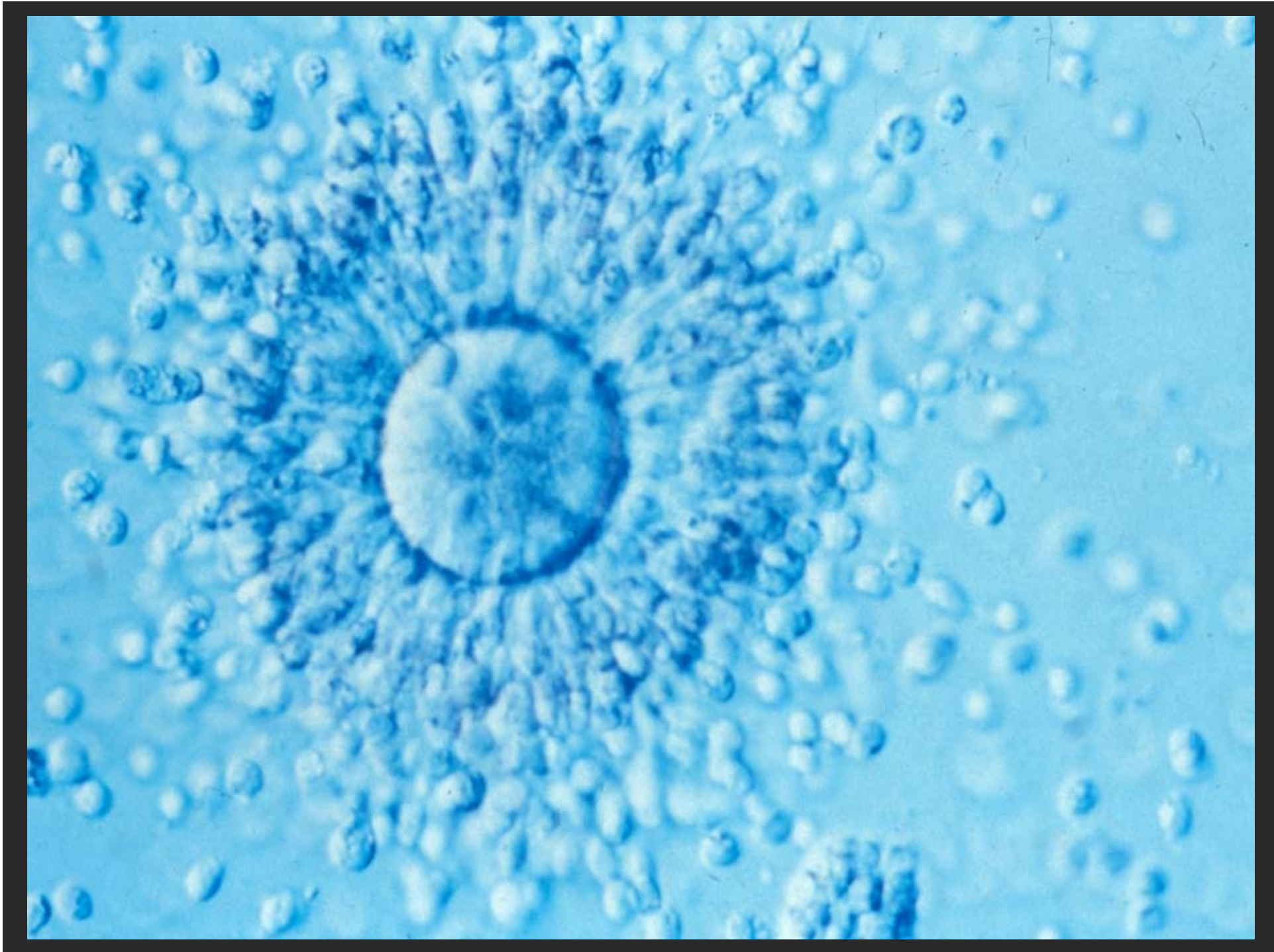
- Pituitary down – regulation with OCP & GNRH agonist (gonadotrophin –releasing hormone)
- Ovarian stimulation with r FSH (follicle stimulating hormone)
- hCG trigger
- Vaginal ultrasound – assisted OPU (ovum pick up)
- Embryo transfer (ET) 2 or 3 days after OPU

- Monitor follicular maturation with vaginal ultrasound
- Aim for cohort of “leading follicles” of 18-20mm diameter
- Average egg No./OPU = 11
- Fertilisation ~60%



IVF Cycle







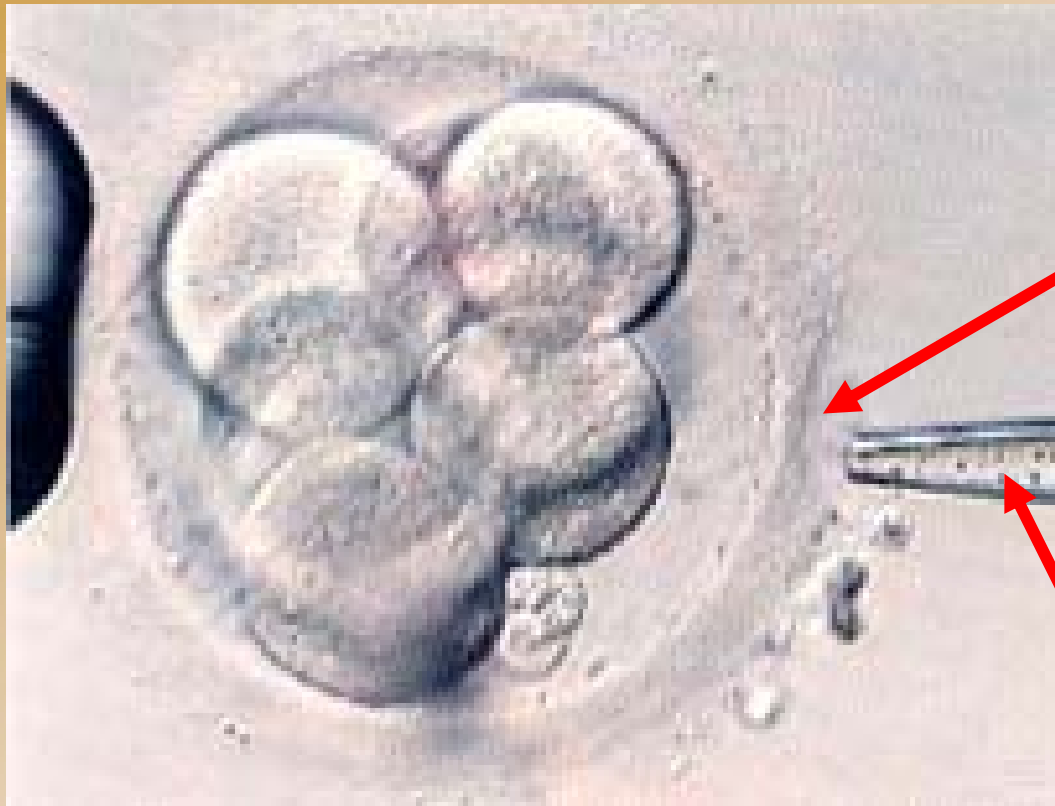








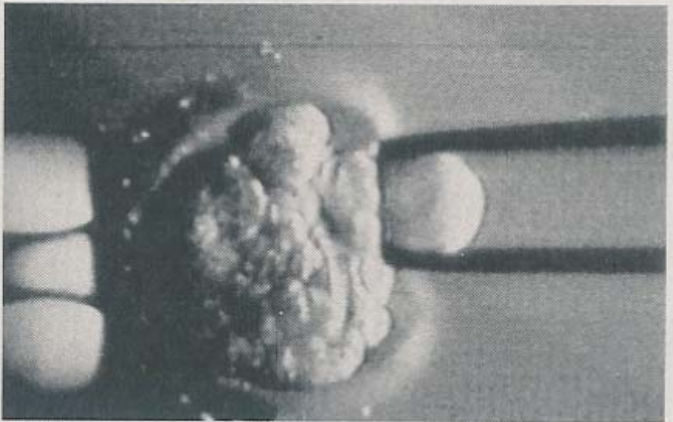
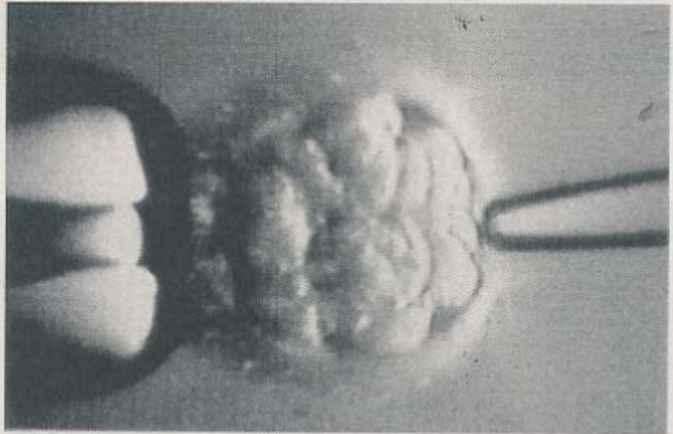
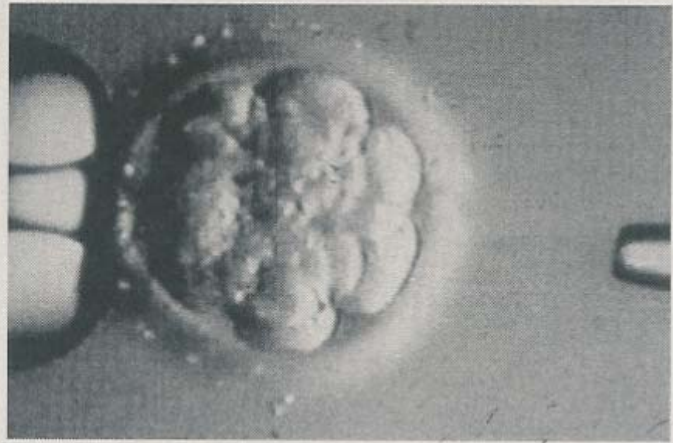
Acid drilling



Hole digested in zona by acid

Pipette loaded with acidified culture media – pH 2.4





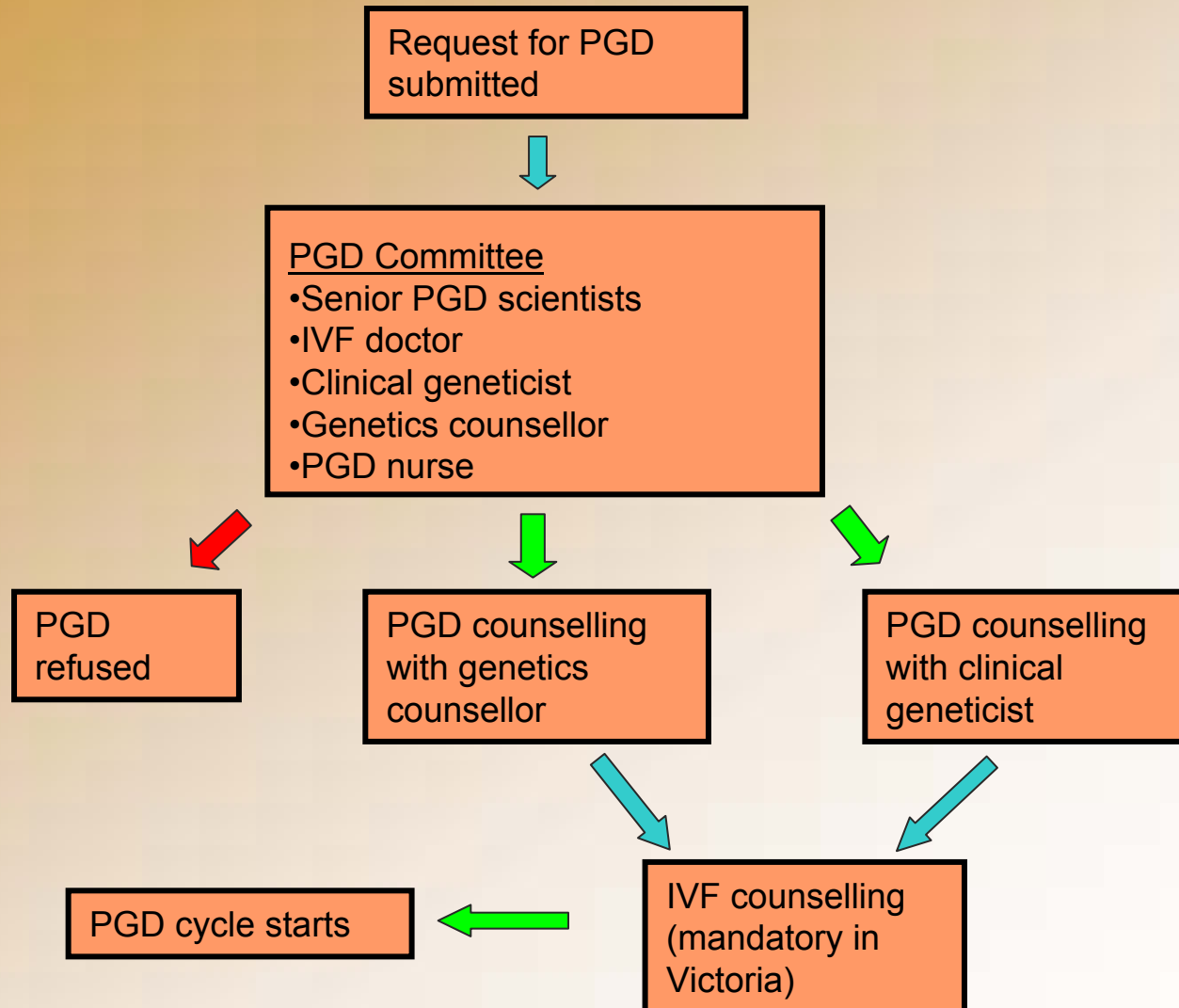
PGD OUTCOMES – “FERTILE” PATIENTS

(all data up to end 2006)

| | SEXING | ROB TRANS | REC TRANS | MONOGENIC | MIVF TOTAL | ESHRE DATA V |
|-------------------|--------|-----------|-----------|-----------|------------|--------------|
| CYCLES | 43 | 29 | 49 | 62 | 183 | 730 |
| AGE | 34.5 | 35.2 | 33.5 | 35.4 | 34.4 | ~34 |
| %“NORMAL” EMBRYOS | 14.3 | 21.4 | 11.5 | 52 | 25.3 | 36.0 |
| % NO ET | 58 | 52 | 51 | 9.7 | 44.2 | 19 |
| % CLIN PREG | 38.9 | 14.3 | 29.2 | 25.0 | 26.8 | 24 |
| IMP. RATE | 34.8 | 13.6 | 28.1 | 18.3 | 21.8 | 16 |



Access to PGD at Melbourne IVF



Consent to PGD

- Although the degree of accuracy of these tests is high, all tests have a failure rate, and the test results could be wrong.
- A full genetic analysis is not being carried out and there are many other genetic conditions that are not being analysed or tested for.
- Finding a normal cell using FISH testing does not mean that a baby resulting from the embryo will have the normal number of chromosomes or be of the expected sex.
- In single gene defect testing, we cannot guarantee that the embryo will not have the disorder being tested for.



Consent to PGD

- It is strongly recommended that all women with PGD pregnancy consider DNA testing in early pregnancy (CVS or amniocentesis) to confirm the early embryo diagnosis.
- Spontaneous conception may occur during a PDG cycle, and all couples having PGD should avoid any form of unprotected sex during the treatment cycle.
- Rarely, some embryos may be destroyed during the biopsy procedure.
- Rarely, it may not be possible to obtain a result on an embryo.
- Embryos that are very poor quality will not be subjected to embryo biopsy and will be discarded.

PGD - BENEFITS

RELIABLE

97% embryos diagnosed

ACCURATE

misdiagnosis rate ~ 2%

RAPID

embryo biopsy and diagnostic testing completed 8 – 30 hours

TREATMENT OPTION

alternative to antenatal testing and TOP



PGD – PITFALLS

- Invasive
- Highly medicalised, requires IVF
- Expensive
- Specific feasibility testing can take months
- No guarantee of pregnancy



HIV AND ASSISTED REPRODUCTIVE TECHNOLOGY

- Chronic Viral Illness Clinic at Royal Women's Hospital Melbourne established 2002
- principle of harm minimisation (reduced risk of HIV transmission to partner and baby)
- Use of assisted reproductive technology (intra-uterine insemination or IVF/ICSI)



HIV +ve MALE

- Good health
- Undetectable viral load for 2 months
- Semen screening for HIV
 - 2 successive samples <50 copies
 - =semen storage for IUI /IVF (all semen samples tested for HIV RNA and DNA)
- risk of transmission to partner <1/2000



CVI PROGRAMME RWH

- 33 referrals
 - 27 male HIV+ve
 - 4 female HIV+ve
 - 1 couple both HIV+ve

- 20 patients treated
 - 16 male HIV+ve - 12 pregnancies
(7 delivered, inc 2 sets twins, 2 ongoing)
 - 3 female HIV+ve - 1 ongoing pregnancy



ACKNOWLEDGEMENTS

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Greta Gillies

Kay Oke

Kate Pope

John McBain

Penelope Foster

Mac Gardner

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Stacey Roe

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Loreto Valent

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Michelle Giles

